Amendments to the Specification

In the Sequence Listing of the printed application please delete pages 1-35 and substitute therefore pages 1-35 of the Sequence Listing included herewith.

Please replace the paragraph bridging pages 4 and 5 of the printed application, with the following paragraph.

More recently assays based on nucleic acid detection have been developed. Probe hybridization assays directed either to RNA or to cDNA have been used to detect non-polio enteroviruses (Rotbart et al., Mol. Cell. Probes 2:65-73 (1988); Rotbart, J. Clin. Microbiol. 28:438-442 (1990); Chapman et al., J. Clin. Microbiol. 28: 843-850 (1990); Hyypia et al., J. Gen. Virol. 70:3261-3268 (1989); Olive et al. J. Gen. Virol. 71:2141-2147 (1990); Gilmaker et al., J. Med. Virol. 38:54-61 (1992); Yang et al., Virus Res. 24:277-296 (1992); Zoll et al., J. Clin. Microbiol. 30:160-165 (1992); Muir et al., J. Clin. Micro. 31:31-38 (1993); Drebot et al., J. Med. Virol. 44:340-347 (1994); Rotbart et al., J. Clin. Microbiol. 32:2590-2592 (1994)). In the absence of nucleic acid sequence information for the non-polio enteroviruses, most of these probes have targeted the highly conserved 5' non-coding region of the viral genomes. Additionally, RNA probes directed to the VP1 capsid gene have been used on a limited basis to identify some of the CBs and a few closely related CAs (Cova et al., J. Med. Virol. 24:11-18 (1988); Alksnis et al., Mol. Cell. Probes 3:103-108 (1989); Petitjean et al., J. Clin. Microbiol. 28:307-311 (1990)). More recently, oligonucleotides having sequences based on the VP4-VP2 junction have been applied as diagnostic and epidemiologic tools (Drebot et al., J. Med. Virol. 44:340-347 (1994); Arola et al., J. Clin. Microbiol. 34:313-318 (1996); Kim et al., Arch. Virol. 142:853-860 (1997); Oberste et al., Virus Res. 58:35-43 (1998)). The sequences in these regions, however, do not always correlate with serotype (Kopecka et al., Virus Res. 38:125-136 (1995); Arola et at., J. Clin. Microbiol. 34:313-318 (1996)). Furthermore, sequences of only certain ~~prototpyes~~ prototypes were available with which to compare and classify clinical samples (Arola et al., (1996)). A generic probe-based assay for nucleic acids in the presence of chaotropic agents is described in U.S. Patent 5,726,012. An assay for a target nucleic acid sequence wherein two separate probes are

hybridized to the same strand of a nucleic acid, and then joined, for example by a polymerase activity, is disclosed in U.S. Patent 5,516,641.

Please replace the paragraph on page 6 of the printed application, lines 17-28, with the following paragraph.

In the case of poliovirus, U.S. Patents 5,585,477 and 5,691,134 to Kilpatrick disclose methods and oligonucleotide primers that are specific and sensitive for detecting all genotypes of poliovirus, as well as primers that are specific and sensitive for distinguishing the three serotypes of poliovirus, and methods for detecting poliovirus and/or distinguishing among the serotypes based on the use of the disclosed primers. Additionally WO 98/14611 discloses an extensive set of degenerate oligonucleotide primers for use in detecting the presence or absence of a non-polio enterovirus in a sample and to identify non-polio enterovirus serotypes. The primers are combined in pairs that detect various groupings of serotypes, and several amplification procedures are carried out in order to detect the presence ~~of~~ or absence of an amplicon in each case. A pooled grid of the results provides information useful in typing a non-polio enterovirus in a sample.

Please replace the paragraph bridging pages 29 and 30 of the printed application with the following paragraph.

In important embodiments of the invention, the primers used are a mixture of oligonucleotides whose use in a PCR amplification provides an amplicon encompassing most or all of the VP1 gene. By way of nonlimiting example, such a mixture may include an oligonucleotide chosen from among an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:9, and a mixture thereof, as well as an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3 (see Table 3); in particularly important embodiments the oligonucleotides employed according to the above mixtures are primer

011 (SEQ ID NO:3), primer 012 (SEQ ID NO:4), and primer 040 (SEQ ID NO:9). The use of either or both of the primers (012, SEQ ID NO:4 and 040, SEQ ID NO:9) provides specific hybridization to target sequences in the 5' region of the VP1 gene of most or all of the non-polio enteroviruses. The third primer, 011 (SEQ ID NO:3), specifically hybridizes to a target sequence in the 2A region of most or all the non-polio enteroviruses. Each ~~of the primers~~ primer is disclosed in PCT application WO 98/14611, which is incorporated herein by reference.

Please replace the paragraph on page 34 of the printed application, lines 6-20, with the following paragraph.

Using these procedures, complete VP1 nucleotide sequences were determined for 57 human non-polio enterovirus strains for which VP1 sequences had not previously been determined. These are summarized in Table 2, which shows both the GenBank accession numbers (numbers AF081293 to AF081349) and the corresponding SEQ ID NOs, 23-79. Forty-seven of the strains were prototype strains for recognized human enterovirus serotypes ~~(Melnick (1996)~~ (Melnick (1996)). The other ten sequenced strains were well-characterized antigenic variants which, while antigenically distinct from their respective prototype strains, were similar enough to them to have been considered to be the same serotype (Committee on Enteroviruses of the National Foundation for Infantile Paralysis, Am. J. Public Health 47:1556-1566 (1957); Melnick (1996)). Combined with the 21 previously available complete enterovirus VP1 sequences, of which 19 are prototypes and 2 are variants, the database constructed for use in the present method includes 66 prototype VP1 sequences and 16 variants or other enteroviruses, including the three poliovirus Sabin strains and the Barty variant of E9.

Please replace the paragraph bridging pages 35-36 of the printed application with the following paragraph. "Design of PCR primers" is underlined in the specification as filed and has not been added to the replacement paragraph.

Design of PCR primers. Since the VP1 sequence was found to correlate with serotype (Example 1), this region was targeted for development of sequence-based molecular diagnostics, namely, generic PCR primers to amplify and sequence a portion of the VP1 gene. Degenerate deoxyinosine-containing PCR primers were designed which specifically recognize regions within or near the termini of the VP1 gene of non-polio enteroviruses. Primers with the broadest specificity within the non-polio enterovirus genus were chosen by searching for regions in the genome that encode amino acid motifs within VP1 and those immediately C-terminal to VP1, in 2A, that are the most conserved across the prototypes. (Echoviruses E22 and E23 were excluded, because it is likely that they will be reclassified as members of a new Picornavirus genus, *Parechovirus* (Mayo et al., J. Gen. Virol. 79:649-657 (1997)). The motif MYVPPG (Met-Tyr-Val-Pro-Pro-Gly) (SEQ ID NO:87) was present in the deduced VP1 amino acid sequences of 44 enterovirus prototype strains whose nucleotide sequences are provided in Example 1. Thirteen prototypes had Ile substituted for Val and CA7 contained Ala instead of Val. CA12, CA14, and EV71 contain the motif, MFVPPG (Met-Phe-Val-Pro-Pro-Gly) (SEQ ID NO:88). In EV68 and 70, a slightly different motif was present, MYVPTG (Met-Tyr-Val-Pro-Thr-Gly) (SEQ ID NO:89). For viruses in the CB-like phylogenetic group the M(Y/F)(V/I)PPG motif is followed by Gly (SEQ ID NO:86), whereas in all other enteroviruses, the motif is followed by Ala (A) (SEQ ID NO:86). To account for differences between the virus groups and for codon degeneracy, two different inosine-containing primers were designed to anneal to this region. Primer 012 (ATGTAYGTICCICCIGGIGG) (SEQ ID NO:4) is based on the amino acid sequence, MYVPPGG (SEQ ID NO:80). Primer 040 (ATGTAYRTICCIMCIGGIGC) (SEQ ID NO: 9) is based on the amino acid sequence, MY(V/I)P(P/T)GA (SEQ ID NO:81). The selectivity of these two primers is primarily due to the first position at the 3' end of each primer (i.e., in primer 012, the base at the 3' end is G, and in primer 040, the base at the 3' end is C) (see Table 3.) In addition, primer 040 contains increased degeneracy at positions 8 and 14 from the 3' end of the primer in order to detect those viruses which encode an isoleucine (position 8) or a threonine (position 14) in these positions. For PCR, primers 012 and 040 were each paired with primer 011 (GCICCIGAYTGITGICCRAA)

(SEQ ID NO:3), which corresponds to the amino acid motif FG(Q/H)QSGA (Phe-Gly-(Gln/His)-Gln-Ser-Gly-Ala; SEQ ID NO:82), present near the 5' end of the 2A gene and which is conserved among most enteroviruses for which the 2A sequence is available.